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U.S. PATENT APPLICATION

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Invention: COMPOUNDS, METHODS AND PHARMACEUTICAL COMPOSITIONS FOR TREATING CELLULAR DAMAGE, SUCH AS NEURAL OR CARDIOVASCULAR TISSUE DAMAGE

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SPECIFICATION

COMPOUNDS, METHODS AND PHARMACEUTICAL COMPOSITIONS FOR TREATING CELLULAR DAMAGE, SUCH AS NEURAL OR CARDIOVASCULAR TISSUE DAMAGE

FIELD OF THE INVENTION

The present invention relates to inhibitors of the nuclear enzyme poly(adenosine 5'-diphospho-ribose) polymerase ["poly(ADP-ribose) polymerase" or "PARP", which is also referred to as ADPRT (NAD:protein (ADP-ribosyl transferase (polymersing)), pADPRT (poly(ADP-ribose) transferase) and PARS (poly(ADP-ribose) synthetase) and provides compounds and compositions containing the disclosed compounds. Moreover, the present invention provides methods of using the disclosed PARP inhibitors to prevent and/or treat tissue damage resulting from cell damage or death due to necrosis or apoptosis; neural tissue damage resulting from, for example, ischemia and reperfusion injury, such as cerebral ischemic stroke, head trauma or spinal cord injury; neurological disorders and neurodegenerative diseases, such as, for example, Parkinson's or Alzheimer's diseases and multiple sclerosis; to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders, such as, for example, myocardial infarction; to treat other conditions and/or disorders such as, for example, age-related muscular degeneration, AIDS and other immune senescence diseases, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes (such as diabetes mellitus), inflammatory bowel disorders (such as colitis and Crohn's disease), acute pancreatitis, mucositis, hemorrhagic shock, splanchnic artery occlusion shock, multiple organ failure (such as involving any of the kidney, liver, renal, pulmonary, retinal, pancreatic and/or skeltal muscle systems), acute autoimmune thyroiditis, muscular dystrophy, osteoarthritis, osteoporosis, chronic and acute pain (such as neuropathic pain), renal failure, retinal ischemia, septic shock (such as endotoxic shock), local and/or remote endothelial cell dysfunction (such are recognized by endo-dependent relaxant responses and up-regulation of adhesion molecules), inflammation and skin aging; to extend the lifespan and proliferative capacity of cells, such as, for example, as general mediators in the generation of oxidants, proinflammatory mediators and/or cytokines, and general mediators of leukocyte infiltration, calcium ion overload, phospholipid peroxidaion, impaired nitric oxide metabolism and/or reduced ATP production; to alter gene expression of senescent cells; or to radiosensitize hypoxic tumor cells.

BACKGROUND OF THE INVENTION

PARP (EC 2.4.2.30), also known as PARS (for poly(ADP-ribose) synthetase), or ADPRT (for NAD:protein (ADP-ribosyl) transferase (polymerising)), or pADPRT (for poly(ADP-ribose) transferase), is a

major nuclear protein of 116 kDa. It is present in almost all eukaryotes. The enzyme synthesizes poly(ADP-ribose), a branched polymer that can consist of over 200 ADP-ribose units from NAD. The protein acceptors of poly(ADP-ribose) are directly or indirectly involved in maintaining DNA integrity. They include histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and Ca^{2+}- and Mg^{2+}-dependent endonucleases. PARP protein is expressed at a high level in many tissues, most notably in the immune system, heart, brain and germ-line cells. Under normal physiological conditions, there is minimal PARP activity. However, DNA damage causes an immediate activation of PARP by up to 500-fold. Among the many functions attributed to PARP is its major role in facilitating DNA repair by ADP-ribosylation and therefore co-ordinating a number of DNA repair proteins. As a result of PARP activation, NAD levels significantly decline. While many endogenous and exogenous agents have been shown to damage DNA and activate PARP, peroxynitrite, formed from a combination of nitric oxide (NO) and superoxide, appears to be a main perpetrator responsible for various reported disease conditions in vivo, e.g., during shock and inflammation

Extensive PARP activation leads to severe depletion of NAD in cells suffering from massive DNA damage. The short life of poly(ADP-ribose) (half-life < 1 min) results in a rapid turnover rate. Once poly(ADP-ribose) is formed, it is quickly degraded by the constitutively active poly(ADP-ribose) glycohydrolase (PARG), together with phosphodiesterase and (ADP-ribose) protein lyase. PARP and PARG form a cycle that converts a large amount of NAD to ADP-ribose. In less than an hour, over-stimulation of PARP can cause a drop of NAD and ATP to less than 20% of the normal level. Such a scenario is especially detrimental during ischaemia when deprivation of oxygen has already drastically compromised cellular energy output. Subsequent free radical production during reperfusion is assumed to be a major cause of tissue damage. Part of the ATP drop, which is typical in many organs during ischaemia and reperfusion, could be linked to NAD depletion due to poly(ADP-ribose) turnover. Thus, PARP or PARG inhibition is expected to preserve the cellular energy level to potentiate the survival of ischaemic tissues after insult.

Poly(ADP-ribose) synthesis is also involved in the induced expression of a number of genes essential for inflammatory response. PARP inhibitors suppress production of inducible nitric oxide synthase (iNOS) in macrophages, P-type selectin and intercellular adhesion molecule-1 (ICAM-1) in endothelial cells. Such activity underlies the strong anti-inflammation effects exhibited by PARP inhibitors. PARP inhibition is able to reduce necrosis by preventing translocation and infiltration of neutrophils to the injured tissues. (Zhang, J. "PARP inhibition: a novel approach to treat ischaemia/reperfusion and inflammation-related injuries", Chapter 10 in Emerging Drugs (1999) 4: 209-221 Ashley Publications Ltd., and references cited therein.)

PARP production is activated by damaged DNA fragments which, once activated, catalyzes the attachment of up to 100 ADP-ribose units to a variety of nuclear proteins, including histones and PARP itself. During major cellular stresses the extensive activation of PARP can rapidly lead to cell damage or death through depletion of energy stores. As four molecules of ATP are consumed for every molecule of NAD (the source of ADP-ribose and PARP substrate) regenerated, NAD is depleted by massive PARP activation and, in the efforts to re-synthesize NAD, ATP may also be depleted.

It has been reported that PARP activation plays a key role in both NMDA- and NO-induced neurotoxicity. This has been demonstrated in cortical cultures and in hippocampal slices wherein prevention of toxicity is directly correlated to PARP inhibition potency (Zhang et al., "Nitric Oxide Activation of Poly(ADP-Ribose) Synthetase in Neurotoxicity", Science, 263:687-89 (1994) and Wallis et al., "Neuroprotection Against Nitric Oxide Injury with Inhibitors of ADP-Ribosylation", NeuroReport, 5:3, 245-48 (1993)). The potential role of PARP inhibitors in treating neurodegenerative diseases and head trauma has thus been recognized even if the exact mechanism of action has not yet been elucidated (Endres et al., "Ischemic Brain Injury is Mediated by the Activation of Poly(ADP-Ribose)Polymerase", J. Cereb. Blood Flow Metabol., 17:1143-51 (1997) and Wallis et al., "Traumatic Neuroprotection with Inhibitors of Nitric Oxide and ADP-Ribosylation, Brain Res., 710:169-77 (1996)).

Similarly, it has been demonstrated that single injections of PARP inhibitors have reduced the infarct size caused by ischemia and reperfusion of the heart or skeletal muscle in rabbits. In these studies, a single injection 3-amino-benzamide (10 mg/kg), either one minute before occlusion or one minute before reperfusion, caused similar reductions in infarct size in the heart (32-42%) while 1,5-dihydroxyisoquinoline (1 mg/kg), another PARP inhibitor, reduced infarct size by a comparable degree (38-48%). Thiemermann et al., "Inhibition of the Activity of Poly(ADP Ribose) Synthetase Reduces Ischemia-Reperfusion Injury in the Heart and Skeletal Muscle", Proc. Natl. Acad. Sci. USA, 94:679-83 (1997). These results make it reasonable to suspect that PARP inhibitors could salvage previously ischemic heart or skeletal muscle tissue.

PARP activation can also be used as a measure of damage following neurotoxic insults following over-exposure to any of glutamate (via NMDA receptor stimulation), reactive oxygen intermediates, amyloid β - protein, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its active metabolite N-methyl-4-phenylpyridine (MPP^+), which participate in pathological conditions such as stroke, Alzheimer's disease and Parkinson's disease. Zhang et al., "Poly(ADP-Ribose) Synthetase Activation: An Early Indicator of Neurotoxic DNA Damage", J. Neurochem., 65:3, 1411-14 (1995). Other studies have continued to explore the role of PARP activation in cerebellar granule cells in vitro and in MPTP neurotoxicity. Cosi et al., "Poly(ADP-Ribose) Polymerase (PARP) Revisited. A New Role for an Old Enzyme: PARP Involvement in Neurodegeneration and PARP Inhibitors as Possible Neuroprotective Agents", Ann. N. Y. Acad. Sci., 825:366-79 (1997); and Cosi et al., "Poly(ADP-Ribose) Polymerase Inhibitors Protect Against MPTP-induced Depletions of Striatal Dopamine and Cortical Noradrenaline in C57B1/6 Mice", Brain Res., 729:264-69 (1996). Excessive neural exposure to glutamate, which serves as the predominate central nervous system neurotransmitter and acts upon the N-methyl-D-aspartate (NMDA) receptors and other subtype receptors, most often occurs as a result of stroke or other neurodegenerative processes. Oxygen deprived neurons release glutamate in great quantities during ischemic brain insult such as during a stroke or heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors, which open ion channels and permit uncontrolled ion flow (e.g., Ca^{2+} and Na^+ into the cells and K^+ out of the cells) leading to overstimulation of the neurons. The over-stimulated neurons secrete more glutamate, creating a

feedback loop or domino effect which ultimately results in cell damage or death via the production of proteases, lipases and free radicals. Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions including epilepsy, stroke, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, schizophrenia, chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Glutamate exposure and stimulation has also been implicated as a basis for compulsive disorders, particularly drug dependence. Evidence includes findings in many animal species, as well as in cerebral cortical cultures treated with glutamate or NMDA, that glutamate receptor antagonists (i.e., compounds which block glutamate from binding to or activating its receptor) block neural damage following vascular stroke. Dawson et al., "Protection of the Brain from Ischemia", Cerebrovascular Disease, 319-25 (H. Hunt Batjer ed., 1997). Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind and hence finding an effective mix of antagonists or universal antagonist to prevent binding of glutamate to all of the receptor and allow testing of this theory, has been difficult. Moreover, many of the compositions that are effective in blocking the receptors are also toxic to animals. As such, there is presently no known effective treatment for glutamate abnormalities.

The stimulation of NMDA receptors by glutamate, for example, activates the enzyme neuronal nitric oxide synthase (nNOS), leading to the formation of nitric oxide (NO), which also mediates neurotoxicity. NMDA neurotoxicity may be prevented by treatment with nitric oxide synthase (NOS) inhibitors or through targeted genetic disruption of nNOS in vitro. Dawson et al., "Nitric Oxide Mediates Glutamate Neurotoxicity in Primary Cortical Cultures", Proc. Natl. Acad. Sci. USA, 88:6368-71 (1991); and Dawson et al., "Mechanisms of Nitric Oxide-mediated Neurotoxicity in Primary Brain Cultures", J. Neurosci., 13:6, 2651-61 (1993), Dawson et al., "Resistance to Neurotoxicity in Cortical Cultures from Neuronal Nitric Oxide Synthase-Deficient Mice", J. Neurosci., 16:8, 2479-87 (1996), Iadecola, "Bright and Dark Sides of Nitric Oxide in Ischemic Brain Injury", Trends Neurosci., 20:3, 132-39 (1997), Huang et al., "Effects of Cerebral Ischemia in Mice Deficient in Neuronal Nitric Oxide Synthase", Science, 265:1883-85 (1994), Beckman et al., "Pathological Implications of Nitric Oxide, Superoxide and Peroxynitrite Formation", Biochem. Soc. Trans., 21:330-34 (1993), and Szabó et al., "DNA Strand Breakage, Activation of Poly(ADP-Ribose) Synthetase, and Cellular Energy Depletion are Involved in the Cytotoxicity in Macrophages and Smooth Muscle Cells Exposed to Peroxynitrite", Proc. Natl. Acad. Sci. USA, 93:1753-58 (1996).

It is also known that PARP inhibitors, such as 3-amino benzamide, affect DNA repair generally in response, for example, to hydrogen peroxide or gamma-radiation. Cristovao et al., "Effect of a Poly(ADP-Ribose) Polymerase Inhibitor on DNA Breakage and Cytotoxicity Induced by Hydrogen Peroxide and γ-Radiation," Terato., Carcino., and Muta., 16:219-27 (1996). Specifically, Cristovao et al. observed a PARP-dependent recovery of DNA strand breaks in leukocytes treated with hydrogen peroxide.